In the sham-operated controls the corresponding values were 7, 7, and 4 days. Thus, while the response to the second-set grafts was accelerated, there was an apparent slight delay in this response relative to the controls. The primary C3H skin grafts are still retained at the time of writing (more than 150 days) by five out of ten of the thymectomized recipients; one died (46 days) with an intact graft while four rejected their grafts. In contrast, all the sham-operated controls vigorously rejected their C3H grafts in 7 days. It follows, therefore, that the thymectomized mice were still immunologically impaired 86 days after irradiation.

Similar results were obtained with CBA mice (group II, Fig. 1). It is noteworthy that eight out of nine of the C3H grafts, which share the $H2^k$ histocompatibility locus with the CBA host, were retained indefinitely. Thus, the response to homografts sharing the H2 locus appears to be more markedly suppressed than that towards grafts not sharing the H2 locus, in this case, BALB/c ($H2^d$). Likewise, CBA grafts were accepted by all seven C3H recipients (group III); three of the mice died with intact CBA grafts (at 171 to 203 days) and four have maintained them for over 212 days. The permanency of the impairment is illustrated best by group III (Fig. 1) in which A-strain grafts, in addition to the CBA grafts, have been maintained by several recipients indefinitely; these mice also showed an impaired response to additional primary homografts implanted as late as 223 days after irradiation.

The effect of thymectomy before or after sensitization was then studied. Adult $LAF_1$ mice were thymectomized and then sensitized by means of two successive grafts of both C3H and rat skin. When these sensitized mice were subjected to x-irradiation (880 rad), injected with syngeneic bone marrow, and challenged with C3H and rat skin grafts 24 days later, there was no difference in the mean survival times between them and the survival times of the sham-operated controls (Fig. 1, group IV). Similarly, when mice were sensitized by two successive grafts of C3H and rat skin and were subsequently thymectomized and irradiated, the mean survival times for the test C3H and rat grafts (engrafted 24 days after irradiation and marrow infusion) were similar to those of the sham-operated controls. Therefore, it appears that thymectomy before or after sensitization of the adult mouse does not alter its responsiveness to skin grafts after irradiation. On the other hand, first-set BALB/c grafts, engrafted on these specifically sensitized thymectomized mice 24 days after irradiation showed a mean survival time of 35, while the value for the controls was 18, indicating that the primary response of these thymectomized mice was impaired.

From our results we can deduce the following. (i) In thymectomized-irradiated adult mice an accelerated response toward second-set allogeneic or xenogeneic skin grafts can occur. (ii) The effect of thymectomy under these conditions is related to the degree of antigenic disparity between the respective skin donors and hosts; that is, the greatest degree of impairment of the homograft response occurs when donor and host are most closely related: non-H2 difference > H2 difference > interspecific difference. A similar differential effect between homografts sharing H2 antigens on the one hand, and differing at the H2 locus, on the other, has been noted by Martinez *et al.* (*1*) in thymectomized neonates. (iii) The unresponsiveness of these mice is long-lasting (probably permanent) and apparently the immunologic functions of the thymus, whatever their nature, cannot be adequately assumed by peripheral cells or tissues in the irradiated mouse. (iv) Thymectomy does not appear to interfere with the induction or maintenance of a state of sensitivity induced in unirradiated adult mice; nor does it interfere markedly with the state of sensitivity in the irradiated mouse once that state has been established.

It is of interest that the thymectomized-irradiated mice in the present study, observed for as long as 267 days after irradiation and bone marrow treatment, have not shown obvious signs of a wasting syndrome. This is in contrast with observations in thymectomized neonates (*2*).

Tyan *et al.* (*4*) showed earlier that thymectomy does not depress xenogeneic graft rejection in irradiated adult mice. We used the same test system and our results are in agreement. However, recent preliminary data indicate that even xenogeneic skin graft rejection can be attenuated in thymectomized-irradiated recipients, when lymph node cells derived from thymectomized-irradiated marrow-restored donors (in contrast with sham-thymectomized controls) were transferred adoptively (*6*). The nonspecific immunologic depression produced by thymectomy in adult x-irradiated mice should not be considered analogous to the state of specific immunological tolerance induced in intact irradiated adult mice or neonatal mice by the injection of allogeneic bone marrow or spleen cells (*7*, *8*).

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8. It is recognized that thymic remnants remaining after surgery could significantly influence these data and their interpretation. Therefore an evaluation of the completeness of thymectomy is being done on each mouse as it dies.
9. Studies reported here were supported by funds from the Bureau of Medicine and Surgery, U.S. Navy.

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## Inhibition of 19S Antibody Synthesis by 7S Antibody

Abstract. *A brief course of treatment with 6-mercaptopurine prevented conversion of 19S to 7S antibodies during the immune response of rabbits to bovine gamma globulin. Specific reactive homologous 7S antibodies given intravenously to animals with such incompletely developed humoral reactions led to an abrupt fall in 19S antibodies. After a lag period the synthesis of 7S antibodies was apparently normal. These results suggest that the concentration of 7S antibodies controls the synthesis of 19S antibodies.*

The administration of the antimetabolite 6-mercaptopurine (6-MP) can lead to the complete suppression of several types of immune responses in experimental animals and man (*1*). When given in sub-suppressive doses (Fig. 1), this compound blocked the normal maturation of the humoral antibody response (*2*). Six normal rabbits were injected in the hind footpads with alum-precipitated bovine γ-glob-

ulin and their antibody responses were measured with the passive hemagglutinin technique. The 19*S* and 7*S* antibodies were distinguished on the basis of sensitivity to 2-mercaptoethanol (2-ME) and by sucrose-gradient ultracentrifugation. As had been demonstrated (*3*), the primary immune response evolved in two distinct phases; initially the antibody was a 19*S* macroglobulin and after approximately 3 weeks only 7*S* antibody was found. Both 19*S* and 7*S* antibodies were present in the period between these extremes of the reaction. When this experiment was repeated in eight rabbits given 10 mg of 6-mercaptopurine per kilogram of body weight subcutaneously daily for 7 days the normal conversion of 19*S* to 7*S* antibody was not found. Instead the production of 19*S* antibody continued for at least 50 days, while 7*S* antibody appeared in small amounts at a titer almost always less than that of the 19*S* titer. It is thus apparent that 6-mercaptopurine given in the dosage indicated arrested the full development of the humoral immune response and that antibody synthesis remained "locked" in its early phase.

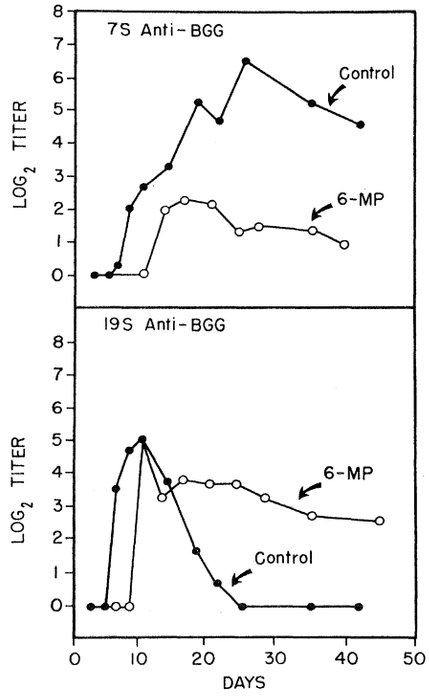


Fig. 1. Antibody responses to bovine γ-globulin in normal and 6-mercaptopurine-treated rabbits. In all experiments, differentiation between 19*S* and 7*S* antibodies was made on the basis of 2-mercaptoethanol sensitivity and sucrose-gradient ultracentrifugation. The reduction in 7*S* antibodies and the sustained production of 19*S* antibodies in the drug treated animals is noteworthy.

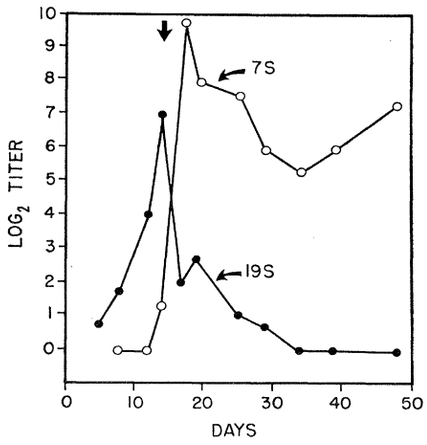


Fig. 2. Inhibition of 19*S* antibodies to bovine γ-globulin by intravenously administered homologous 7*S* antibodies to bovine γ-globulin (arrow) in 6-mercaptopurine-treated rabbits. Each point represents an average of six experiments.

The following experiments utilized this phenomenon in order to explore factors which might control the sequential appearance of 19*S* and 7*S* antibodies. A series of rabbits were injected with alum-precipitated bovine γ-globulin (2 mg in the hind footpads) and also were given 10 mg of 6-mercaptopurine per kilogram of body weight subcutaneously daily for 7 days beginning on the day of antigen injection. Serum antibodies were titrated by the tanned–red blood cell method (*4*). The sensitivity to 2-mercaptoethanol (*5*) was determined by incubating a 1:5 dilution of the serum with an equal volume of 0.2*M* 2-mercaptoethanol at 37°C for 30 minutes. The "19*S*" titer was considered equal to that titer of antibody inhibited by the 2-mercaptoethanol. Representative serums were also analyzed after sucrose-gradient ultracentrifugation in a Spinco Model 2 preparatory ultracentrifuge (*6*). No discrepancies between the presence of 2-mercaptoethanol-sensitive antibody and the ultracentrifuge data were found. As an additional check, a representative sample was subjected to starch-block electrophoresis (*7*). The 2-mercaptoethanol-sensitive antibody that appeared near the bottom of the ultracentrifuged tube was found in the electrophoretic region of $\gamma_1$-globulin, while the 2-mercaptoethanol-insensitive antibody that was found in the upper third of the ultracentrifuged tube was recovered from the $\gamma_2$-globulin zone of the starch block. For the sake of convenience, the 2-mercaptoethanol sensitive antibody will be referred to as "19*S*" and the 2-mercaptoethanol-insensitive antibody as "7*S*." These 6-mercaptopurine-treated rabbits elaborated immune responses similar to those depicted in Fig. 1; uninhibited 19*S* antibody synthesis in the presence of markedly diminished production of 7*S* antibody. They were then utilized in the following experiments.

Since it was possible that the concentration of 7*S* antibody controlled the synthesis of 19*S* antibody, perhaps in a feedback mechanism, six rabbits were infused with homologous rabbit serum containing large amounts of 7*S* antibody and no detectable 19*S* antibody. The tanned–red blood cell titer of this serum was $2 \times 10^6$, and each rabbit received approximately 4 ml intravenously (Fig. 2). There was a prompt decrease in 19*S* antibodies after the administration of the 7*S* antibodies. Concomitantly, the 7*S* antibody increased and, after a gradual fall, increased once again. Preliminary data indicate that one-half of the 19*S* antibody present before the infusion of homologous 7*S* antibody had disappeared in approximately 2 days. This value is equivalent to the biological half-life of rabbit $\gamma_1$-globulin (*8*) and suggests that the synthesis of 19*S* antibodies ceased within 24 hours of the administration of the 7*S* antibody solution.

The specificity of this response was tested in rabbits treated with 6-mercaptopurine and immunized with bovine γ-globulin by the intravenous administration of a rabbit antiserum containing high titers of 7*S* antibodies to egg albumin. In no instance did the amount of 19*S* antibodies fluctuate significantly after the injection of this antibody, which was not cross-reacting.

Rabbit serum containing 7*S* antibodies to bovine γ-globulin was added to this antigen at equivalence, and the resulting precipitate was washed and suspended in saline. This preparation failed to cause any change in the amounts of 19*S* or 7*S* antibodies to bovine γ-globulin as determined by the use of the 2-mercaptoethanol inhibition and sucrose-gradient techniques in rabbits treated with 6-mercaptopurine and immunized with bovine γ-globulin.

The mechanism of the conversion of

19*S* to 7*S* antibodies during the humoral immune response is unknown. The present results indicate that it can be blocked by 6-mercaptopurine and in such a "blocked" preparation the normal sequence of events is promptly restored by the administration of specific, reactive 7*S* antibody. How the 7*S* antibody acts in this manner is unknown and our data do not permit a choice among several possibilities. The advantage of using the 6-mercaptopurine-treated rabbit in the study of this conversion is that the early phases of this sequence are highly exaggerated and appear to be suspended in time. Thus, ample opportunities are available for attempting to modify or restore the sequence.

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## Alkylation of Synthetic Polynucleotides

Abstract. *The synthetic polynucleotides may be used as models for the effects of alkylating agents on nucleic acid. Alkylation of polyadenylic acid with methyl methanesulfonate yields a polymer methylated in the one-position of adenine. This alteration in structure produces marked changes in physical properties and decreases the capacity to code for polylysine synthesis.*

There have been numerous studies of the effects of alkylating agents on nucleic acids and on their constituent bases, nucleosides, and nucleotides. Reiner and Zamenhof (*1*) and Lawley and Wallick (*2*) have isolated derivatives of guanine after the treatment of isolated deoxyribonucleic acids with dimethyl sulfate. Lawley and Brookes (*3*) isolated other derivatives of guanine, as well as derivatives of adenine, cytosine, and uracil, after similar reactions. Extensive studies of the methylation of purine nucleosides have been reported recently by Jones and Robins (*4*); their paper also reviews the occurrence of methylated bases in nucleic acids.

A systematic study of the effect of alkylating agents on synthetic polynucleotides, however, can provide much additional information on the mode of action of these compounds. Through such studies, it is possible to obtain more definite information on the nature and cause of changes in the physical properties of the polymers, as well as direct information on the biological effects of alkylation. Thus, synthetic polynucleotides that code for protein synthesis can be alkylated, and the resulting effects on their coding properties can be determined. In this paper, we report studies of this type on the methylation of polyadenylic acid.

Polyadenylic acid obtained commercially was further purified by phenol extraction, and exhaustive dialysis against 0.05*M* NaCl–0.015*M* sodium citrate solution, 0.01*M* NaCl, and glass-distilled water. Alkylations were carried out at 20°C and *p*H 7; the *p*H was maintained in the absence of buffer by an automatic titrimeter. Methyl methanesulfonate ($MeMeSO_3$) was used as alkylating agent, and the extent of alkylation was controlled by varying concentration of reagents and time of reaction (Table 1). At the termination of the reaction, cacodylate buffer (*p*H 7) was added to an ionic strength of 0.2, and the polymer was precipitated by the addition of four volumes of cold 95 percent ethanol. The precipitate was washed with ethanol, redissolved in glass-distilled water, and lyophilized.

Methylation occurred in the 1-position of adenine, as shown by the identification of 1-methyl adenine in the acid-hydrolysate. The polymer was dissolved in 1*N* HCl at a concentration of 10 mg/ml and heated in a sealed tube at 100°C for ½ hour. The hydrolysate from this treatment was chromatographed on Whatman No. 1 filter paper in the two descending solvent systems that were employed by Brookes and Lawley (*6*). Only one derivative was detected, although two faint additional spots were found in methylated polymer No. 3 after brief periods of hydrolysis. The derivative had a mobility, relative to adenine, of 1.3 in solvent A (methanol, concentrated HCl, water, 7:2:1) and of 0.7 in solvent B (*n*-butanol saturated with water-aqueous ammonia, 100:1), as compared to values of 1.3 and 0.8 found by Brookes and Lawley for 1-methyladenine.

Table 1. Methylation of polyadenylic acid.

| Methylated polymer No. | Poly A (mg/ml) | $MeMeSO_3$ (mg/ml) | Reaction time (min) |
|---|---|---|---|
| 1 | 1.33 | 6.2 | 66 |
| 2 | 1.22 | 9.7 | 186 |
| 3 | 1.10 | 25.3 | 470 |

Separate portions of the acid hydrolysates were chromatographed in solvent A on Whatman filter paper No. 40, and both adenine and derivative spots were eluted in cacodylate buffer (*p*H 7, ionic strength 0.2). Spectra determined in this solvent on a Beckman DU spectrophotometer against appropriate paper blanks agreed closely with the spectrum of 1-methyladenine, as presented by Brookes and Lawley. The percentage of adenine substituted in each polymer was calculated on the assumption that the molar absorptions of adenine and 1-methyladenine are equal at 260 mμ. These percentages are shown in Table 2.

The percentage of hypochromicity at 260 mμ, which reflects the degree of secondary structure in a general way, was determined by alkaline hydrolysis in the following manner. Stock solutions of polymer were prepared at room temperature in glass-distilled water at a concentration of 0.5 mg of polymer per milliliter; these were agitated gently for 4 hours and then centrifuged at 3000*g* to remove particulate matter, although no polymer gel was visible. Duplicate portions (0.11 ml) of each polymer were (i) hydrolyzed with 1 ml of 1.1*N* NaOH at 37°C for 14 hours and (ii) diluted to 5 ml in cacodylate buffer (*p*H 7.0, ionic strength 0.2) for immediate determination of absorption at 260 mμ. Base hydrolysates were neutralized, diluted in the same fashion, and com-